Physicochemical Properties of Cheese Produced from Nigeria Dwarf Goat Milk, Cow Milk and Blends Using *Brevibacterium linens* as Coagulant.

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ABSTRACT

This study investigated the physicochemical properties of cheese produced from Nigeria dwarf goat milk, cow milk and their blends using *B.linens* as coagulant. *Brevibacterium linens* were isolated from samples of milk. Milks were filtered and pasteurized at 90 ± 1 ºC for 10 min followed by direct acidification with *Brevibacterium linens* . The vats were incubated at 36 ºC and gel was pressed, drained, cut, salted and package. The samples were analyzed for physicochemical properties using standard laboratory procedures. All the cheeses produced from *B. linens* as coagulant was significantly (P > 0.05) different when compared with control. The mean value for moisture contents, fats, carbohydrate, proteins ash, pH, TSS, TTA and yield were; 52.01-43.22%, 19.04-17.82%, 62.01-56.04%, 14.01-12.33%, 2.35-1.18%, 1.18-1.14%, 6.85-6.25%, 0.74-0.68%, 28.20-24.35% respectively. The study revealed that, the cheese produced from goat milk has higher protein content followed by blends of goat and cow milk and there was no significant different (p<0.05) in both cow milk and control sample. Therefore, Efforts should therefore be intensified toward commercial production of cheese and other dairy products using *Brevibacterium linens,* as a coagulant starter culture.

Keywords: Physicochemical, Nigeria dwarf goat milk, cow milk, cheese, *Brevibacterium linens*

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1. BACKGROUND OF RESEARCH

Cheese is one of the most popular milk products, which is most commonly produced from raw or pasteurized cow's milk, but also from other species such as sheep and goats (Bennett & Johnston, 2004; Johnson, 2017; Khattab *et al*., 2019), it can differ from each other by their making process, ripening time (if applied), type of milk used, texture, color, flavor, microbial counts and diversity, coagulation type (enzymatic and/or acid)… etc (Kamimura *et al*., 2019). With respect to the relevant literature, the use of raw milk for the production of cheese has advantages and disadvantages. Raw milk cheeses tend to display greater variability in comparison to their counterparts made of pasteurized milk and they are characterized by a strong and unique organoleptic profile. This profile, sometimes, is highly appreciated by the consumers, as in the case of raw cows' milk cheese (Beuvier et al., 1997; Montel et al., 2014), or not always gaining the consumers' sensory acceptance, as in the case of raw goats' milk cheese

(Mituniewicz–Małek et al., 2019). Cheeses from raw milk possess also some healthy qualities, since studies in populations with a similar genetic background have shown that children growing up on a farm have a lower risk of developing asthma and allergies due to the consumption of raw unpasteurized milk (Waser et al., 2007). This is especially true for those who consume goat milk, which has been identified as having more favourable allergenic characteristics (Ranadheera et al., 2019; Verruck et al., 2019). Several studies have shown that cheese produced from raw milk contains a wide variety of microflora, including beneficial bacteria, especially lactic acid bacteria, which contribute to a more intense and stronger flavor production than that of pasteurized milk cheeses (Casalta et al., 2009; Grappin & Beuvier, 1997).

These results have been attributed to several indigenous microbiota, such as Lactococcus spp., Lactobacillus spp., Leuconostoc spp., andEnterococcus spp. Moreover, indigenous microflora, especially lactic acid bacteria, can control the proliferation of many contaminating bacterial pathogens and thus protect the cheeses from microbiological risk, making raw milk cheeses superior in terms of microbiological safety, in comparison to cheeses made from pasteurized milk (Yoon et al., 2016).

Brevibacterium linens has long been recognized as an important dairy microorganism because of its ubiquitous presence on the surface of a variety of smear surface-ripened cheese such as Limburger, Munster, Brick, Tilsiter and Appenzeller (Motta and Brandelli,2008). The growth of *B.linens* on the surface is thought to be an essential prerequisite for the development of the characteristic colour, flavor and aroma of smear surface-ripened cheeses (Ades and Cone, 2009). *Brevibacterium* are of interest to the food industry because they produce amino acids such as glutamic acid which is of use in the production of flavour enhancer such as monosodium glutamate.

They also produce important enzymes used in cheese ripening. *Brevibacterium linens* is the type strain and has a growth temperature range of 8–37 °C and an optimum of 21–23 °C (Motta and Brandelli, 2008). *Brevibacterium* have also been isolated from wheat samples (Legan, 2000).*B.linens* produces red or orange or purple-coloured pigment of aromatic carotenoide type which are not common in other bacteria.

This alcalophilic bacterium is able to produce methanethiol fromL-methionine and tolerate a high NaCl concentration up to 15%, *B.linens* produces antimicrobial substances which inhibits the growth many gram positive food poisoning bacteria as well as several yeasts and moulds. *B. linens* synthesizes highly active and multiple proteolytic enzymes during its growth. In acceleration of cheese ripening process, it is possible to improve flavor and eliminate bitterness with the use of enzymes (peptide) from *B. linens* alone or in combination with commercially available enzymes (Motta and Brandelli, 2008). The contribution of *Brevibacterium* towards cheese production has been under investigation for some time, showing that it can break down lipids and proteins (i.e. casein) with the use of extracellular proteases and lipases,(Rattray and Fox, (1999), Ozturkoglu-Budak *et al*., 2016) .

Many *Brevibacterium* isolates also have the ability to modify sulfur-containing amino acids to produce volatile sulfur compounds which are important for flavor development, (Amarita *et al*., 2004, Yvon *et al*., 2000, Bonnarme, Psoni and Spinnler, (2000)). *Brevibacterium* strains are thus often used as surface-ripening cultures in many different cheese types, (Bockelmann *et al*., 2005). Understanding the functional potential of cheese bacteria is essential in the combined effort with cheese producers to shorten ripening times, reduce spoilage, better control cheese aroma, and increase food safety. Therefore, the aim of this study was investigated the physicochemical of cheese produced from Nigeria dwarf goat milk, cow milk and blends using *Brevibacterium linens* as coagulant.

2.0 MATERIALS AND METHODS

2.1 Source of Milk

Fresh Nigeria dwarf goat milk and cow milk were purchased from National Veterinary Research Institute (Vom) in division of Animal Health and Production Technology, (AHPT), Jos Plateau State, Nigeria. Milk samples were then kept in an ice box immediately after collection. The sample of cheese used as control was purchased from food chemical shop in Jos metropolis.

2.2 Isolation of *Brevibacterium linens* from cheese

Brevibactrium linens were isolated from sample of milks. Prior to isolation of *Brevibacterium linens*, sample of milks were thawed in the dark at 4^{o}C. The smear was collected from cheese, by scraping the surface of the cheese and weighed. The culture was grown in 250ml Erlenmeyer flask containing 50ml of a medium composed of 20g/L D-glucose (Carloerba, London), 5g/L casamino acids (Difco), 1g/L yeast extracts (Biokar), 5g/L NaCl and 1g/L KH_2PO4.

The pH was adjusted to 6.9 and the medium was sterilized at 121^{o}C for 15minutes and incubated at 25^{o}C for 48hours with stirring (150rpm) to oxygenate the medium (Galaup *et al.*, 2005).

2.2.1 Sample preparation

2.2.2Production of cheese

Three different cheese types were made from two samples of fresh milk: CCM (cheese made from cow's milk), CGM (cheese made from goat's milk) and CCGM (cheese made from cow's milk and goat's milk, 1:1 ratio, L:L). The cheeses were produced using the method described by Adetunji and Babatobi, (2011). 500ml of each sample of milks were filtered and pasteurized at 90 ± 1 ^{o}C for 10 min followed by direct acidifying/inoculating with 10ml/l *Brevibacterium linens* .

The vats were incubated at 36 ^{o}C until a firm curd was formed (approximately 40 min). The obtained gel was allowed to drain, press, gently cut into cubes, salted in brine (12 g/L NaCl), placed in perforated rectangular containers (approximate capacity of 250 g) and maintained at 10 ^{o}C under pressure for 4 h and vacuum packaged. The cheese obtained after storage at 10 ^{o}C for 24h was regarded as the final product.

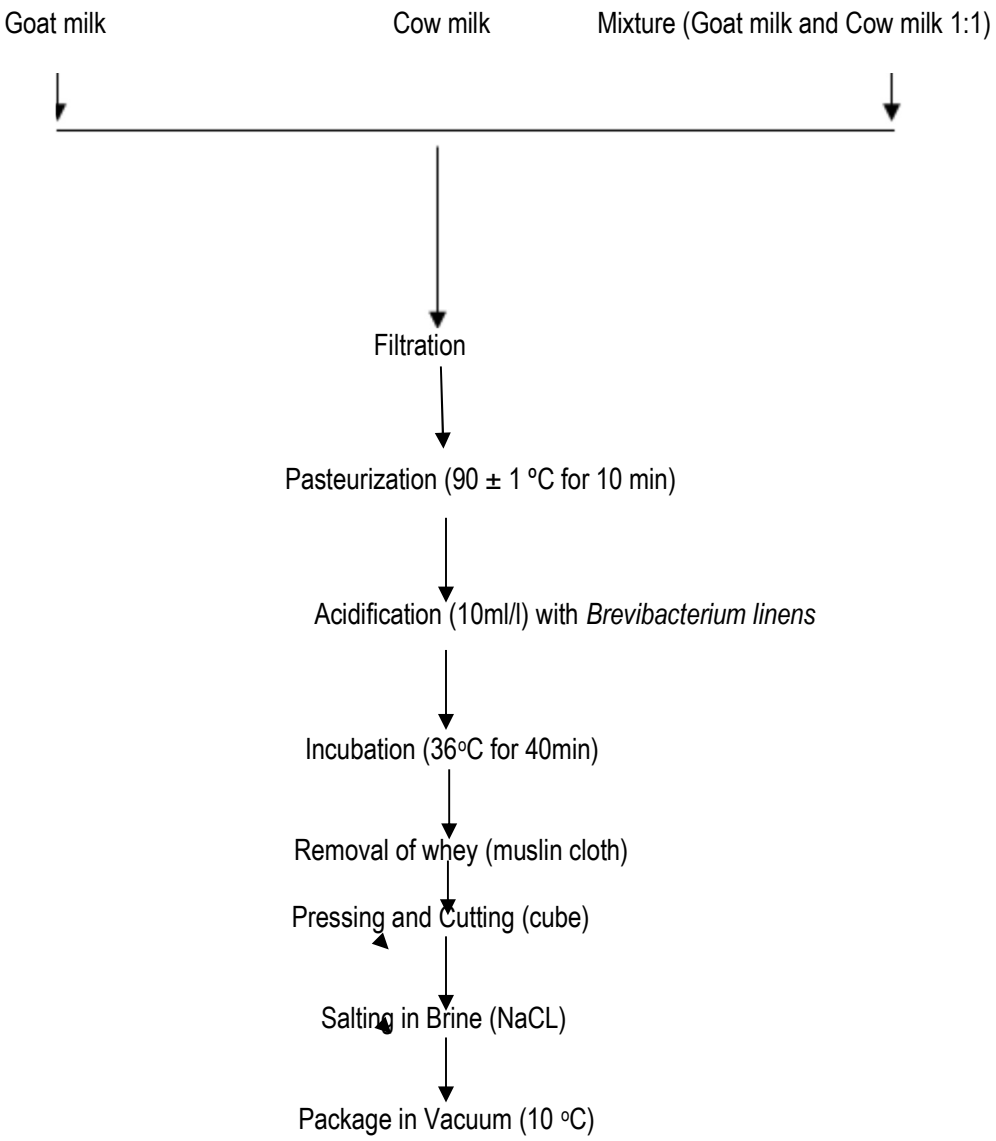

Figure 1: flowchart for the production of goat milk, cow milk and their mixture cheese.

2.3 Determination of proximate composition cheese samples

The moisture, crude protein, crude fat and total ash contents of the cheese samples were determined according to the standard methods of AOAC (2012). The carbohydrate content was determined as shown below:
% Carbohydrate = 100%–(% moisture + %protein + % fat + % ash), (Akume *et al*., 2019).

2.4 Determination of Physical properties of cheese

2.4.1Total Titratable Acidity (TTA)

Total titratable acidity was determined using the AOAC (2005) method. About 10 g of the sample was dissolved in 30 ml of distilled water in a beaker and stirred. The mixture was filtered into 100 ml standard volumetric flask. The filtrate was made up to 100 ml. A 10 ml sample of the filtrate was pipetted into a beaker and 1 drop of phenolphthalein was added. The mixture was titrated against standard 0.01 N Sodium Hydroxide solutions until light pink color was attained. The reading of the burette was recorded.

$$TTA = \frac{N(NaOH) \times \text{titre value} \times \text{lactic acid value} \times \text{dilution factor} \times 100}{10}$$

Where N = Normality of NaOH (0.01)
Lactic acid value = 0.09
Dilution factor = 10

2.4.2 pH Determination

pH was determined using pH meter (Unicam 9450, Cambridge, UK). About 1.0g of the cheese was dissolved in beaker containing 10 ml of distilled water and stirred. The electrode of the pH meter was dipped into the beaker and readings were obtained from the photo-detector on the pH metre.

2.4.3 Total Soluble Solids

This was determined using the AOAC (2005) method. A clean glass dish was dried in an oven (103-105 ^{o}C) until constant weight was achieved, cooled in a desiccators and weighed. About 2.0 g was dissolved in 50 ml distilled water. About 20 ml of filtered water sample was evaporated on a water bath at temperature 90 ^{o}C followed by oven drying at temperature 103 ^{o}C- 105 ^{o}C for about an hour. The glass was cooled in desiccators, reweighed and the increased weigh recorded.

2.4.4 Determination of Percentage Yield

Percentage yield of cheese was determined by method described by Igyor, Igbian, and Iorbo (2006). The yield of cheese from Nigeria dwarf goat milk; cow milk and goat milk and cow milk blends was determined by the calculation as follows:

$$\text{Yield of Cheese (\%)} = \frac{W_2 \times 100\%}{W_1}$$

W1 = Weight (g), goat milk, cow milk and cow-goat milk blend.
W2 = Weight (g), cheese produced.

2.5 Statistical analyses

The data obtained were subjected to Analysis of Variance (ANOVA), while Duncan Multiple range test was used to separate means where significant differences existed, data analyses was achieved using the Statistical Package for Social Statistics (SPSS) software version 20.0. All analyses were performed in triplicate determination.

3.0 RESULTS AND DISCUSSION

3.1 Proximate composition of cheese produced from Nigeria dwarf goat milk, cow milk and blends using *Brevibacterium linens* as coagulant

Table 2: Proximate composition of cheese produced Nigeria dwarf goat, cow cheese and blends

Sample	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	CHO (%)
GC	45.21 ± 0.06	14.01 ±0.04	19.04 ± 0.01	1.18 ± 0.01	56.04 ± 0.02
CC	48.13 ± 1.00	12.50 ± 0.01	18.10 ± 0.04	2.04 ± 0.04	58.10 ± 0.02
BC	52.01 ±0.02	13.19 ± 0.05	18.50 ± 0.06	2.35 ± 0.01	62.01 ± 0.01
CS	43.22 ± 00.5	12.33 ±0.01	17.88 ± 0.04	2.08 ± 0.01	60.02 ±0.01

Values are means ± SD of triplicate determination. GC: Nigeria dwarf cheese, CC: cow cheese, BC: blends of goat-cow cheese, CS: control sample.

Table 1, shows the result of proximate composition of the cheese samples made from Nigeria dwarf goat milk, cow milk, blend of goat-cow milk and control sample (produced from cow milk which coagulated from Sodom apple). Moisture content of the cheese produced from all the cheese samples ranged; 43.22-52.01s%. Moisture content of control sample in which Sodom apple was used as coagulant was significantly ($P < 0.05$) lower than all other samples from *Brevibacteruim linens* as coagulant. The disparities observed in the moisture content of the mixture goat-cow cheese, variation may be attributed to the mixture of their milk. However, moisture content noted from cheese produced from cow milk, was significantly ($P > 0.05$) higher than cheese produced from Nigeria dwarf goat milk. Higher moisture content could favour growth and proliferation of microorganisms; thus reducing the shelf-life of cheese (Orhevba and Taiwo, 2016). There were significant ($P < 0.05$) differences in the protein profile of the studied cheese samples.

Besides the cheese from Nigeria dwarf goat milk was recorded highest followed by blends while cow milk and control sample recorded low protein profile .The disparity seen in the protein content of cheese in this study could probably be due to the denaturization of whey protein during pasteurization and the resulting β-lactoglobulin–κ casein entraps denatured whey proteins, which may lead to some minor differences in amino acid profiles between lactic cheese and soft cheese (Henry *et al*., 2002 cited in Raynal-Ljutovac et al., 2008). This work was in agreement with the contribution of Rattray & Fox, (1999) *Brevibacterium linens* towards cheese production, showing that it can break down lipids and proteins (i.e. casein) with the use of extracellular proteases and lipases,(Ozturkoglu-Budak, et al., 2016).This study also, in line with report of Henning *et al*., (2006) casein remains in the curd, but caseins are low in sulphur-containing amino acids and the nutritional value of cheese protein is slightly lower than that of total milk protein. Also, progressive breakdown of casein during ripening is reported to increase its digestibility (Henning et al., 2006).

Moreover, proteolysis induced by fermentation and ripening increases amounts of bioactive peptides and free amino acids present in the cheese .Fat content of Nigeria dwarf goat cheese was significantly ($P > 0.05$) than all other sample cheeses. The curd contains almost 95 percent of the fat, and during cheese-making the fat is concentrated between 6- and 12-fold, depending on cheese variety (Fox and McSweeney, 2004). Although the content of nutritionally interesting FAs such as CLA can be increased by lipid supplementation of the goat diet, this may be accompanied by a change in cheese flavour (Chilliard and Ferlay, 2004, Chilliard *et al.*, 2005 and Chilliard *et al*., 2006a, cited in Raynal-Ljutovac *et al*., 2008).

The ash content in foodstuff is a measure of mineral elements in food (Balogun *et al* 2016). Cheese samples made from Nigeria dwarf goat milk was significantly ($P < 0.05$) different in ash content than those produced from other milk samples and the control. Carbohydrate value ranged from 56.04 % to 62.01% among the samples, with the highest content recorded for cheese made from blends of goat- cow milk followed by that from control sample. Decrease in carbohydrate content in cheese produced from Nigeria dwarf goat milk due to the lost lactose in cheese production. Whey contains up to 94 percent of the lactose, much of which is lost in cheese making.The remaining lactose is partially transformed into L-lactate or D-lactate (Trujilllo *et al.*, 1999, cited in Raynal-Ljutovac *et al.,* 2008), or into glucose and galactose on cheese-making. These residual carbohydrates found in fresh cheeses disappear with increasing ripening time (Raynal-Ljutovac *et al.,* 2008).

3.2 physical properties of cheese samples

Table 2: Physical properties of cheese samples

Sample	pH (%)	TTA (%)	TSS (%)	Yield (%)
GC	6.82 ± 0.06	0.68 ± 0.01	1.18 ± 0.04	24.35 ± 0.02
CC	6.85 ± 0.02	0.70 ± 0.01	1.14 ± 0.02	26.15 ± 0.02
BC	6.50 ± 0.02	0.74 ± 0.02	1.16 ±0.02	28.20 ± 0.06
CS	6.25 ±0.01	0.72 ± 0.04	1.17 ±0.04	26.02 ± 0.04

Values are means ± SD of triplicate determination. GC: Nigeria dwarf goat cheese, CC: cow cheese, BC: blends of goat-cow cheese, CS: control sample. TTA: Titratable acidity, TSS: Total soluble solid.

The physicochemical properties of milk samples from Nigeria dwarf goat cheese, cow cheese, and their blends and control sample are presented in Table 2. The pH of the samples was ranged from: 6.25%, 6.50%, 6.82% and 6.85%. There was no significant ($p< 0.05$) difference in the pH of all the samples of the cheese. The higher the pH, the lower the TTA and vice versa (Korshina *et al*., 2019). Fresh cow milk typically has a pH between 6.5 and 6.7. As milk goes sour, it becomes more acidic and the pH gets lower. Casein is the most important protein in milk, while the proportion of whey proteins is relatively low (Barlowsk *et al*., 2011). The report of the TTA of the cheese produced from blends of Nigeria dwarf goat milk and cow milk were significantly ($P>0.05$) different from other sample. This may be as a result of microbial proliferation during transportation from where the samples were purchased. The percentage of acid present in milk is a rough indicator of its age (Gemechu *et al*., 2015). Normal fresh milk has an apparent acidity of 0.14 to 0.16% as lactic acid (Siriwardhana and Gunasena, 2018).

The Total Soluble Solids (TSS) content in Nigeria dwarf goat milk was significantly ($P>0.05$) higher than those of cow, blends and control sample respectively. The values reported in this study followed similar trend with those reported by (Hamad *et al*., 2016) who revealed that considerable content of TSS and fat was detected in coconut milk than in cow milk. There was increase in percentage yield of cheese produced from blends of Nigeria dwarf goat milk-cow milk followed by cow cheese and control sample increased in the percentage yield may depends on the level of protein available for curdling by enzymes or acid, and a subsequent decrease in percentage yield 24.35% of Nigeria dwarf goat milk. This result agreed with the work of Weimer, *et al*., (2000), who highlight the positive contribution of this *Brevibacterium linens* to cheese production by accelerating the ripening process, final appearance and quickening maturation of cheese production. However, the decrease in percentage yield of cheese with goat milk and increasing cow milk agreed with the findings of Igyor et al. (2006) who reported a decline in the percentage of cheese yield as the percentage of soy milk inclusion increased in cheese.

4.0 CONCLUSION

The study revealed that, the cheese produced from goat milk has higher protein content followed by blends of goat and cow milk and there was no significant different ($p<0.05$) in both cow milk and control sample. However, of the cheese produced from blends Nigeria dwarf goat milk and cow milk recorded highest percentage of 28.20% and lowest was recorded for goat milk cheese of 24.35% and there was no significant different in cheese produced from cow milk cheese and control ($p<0.05$). Goat milk and cow milk are some of the healthiest beverages that are available today, but goat milk is easy to digest than cow milk because of small fat globules and is naturally homogenized. Goat milk is non-allergic as compared to cow milk and it can be used in the treatment of certain diseases. Efforts should therefore be intensified toward commercial production of cheese and other dairy products using *Brevibacterium linens,* as a coagulant starter culture.

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